L27 ANSWER 36 OF 40 MEDLINE
ACCESSION NUMBER: 89131993 MEDLINE
DOCUMENT NUMBER: 89131993 PubMed ID: 3066165
TITLE: Marrow reticulo-fibroblastoid colonies (CFU-RF derived) spontaneously release an erythroid colony (BFU-E) enhancing factor.
AUTHOR: Izaguirre C A; Ross W M; Hsu E Y
CORPORATE SOURCE: Department of Medicine, University of Ottawa, Canada.
SOURCE: ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY, (1988) 241 303-9.
Journal code: 0121103. ISSN: 0065-2598.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198903
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 19900306
Entered Medline: 19890317

AB The marrow microenvironment is composed of an extracellular matrix as well as a heterogeneous population of cells. **Isolation** of the various cell types and analysis of their function is necessary for a better understanding of their roles in hemopoiesis. We have recently reported a colony assay for a cellular component of the marrow microenvironment. The assay consists of a cellular component of the marrow microenvironment. The assay consists of a plasma clot-methylcellulose marrow culture. The stimulator is PHA-stimulated leukocyte conditioned medium (PHA-LCM) and hydrocortisone (5 x 10(-5)M). The fibrin strands appear to act as a **substrate** for the growth of Reticulo-Fibroblastoid colonies derived from the CFU-RF precursor. RF colonies can be subcultured forming adherent layers when transferred to liquid cultures. Confluent adherent layers can be maintained for long periods of time by changing medium every 3 to 5 days. Supernatants derived from unstimulated RF cultures (RF-CM) were tested for growth promotion of hemopoietic precursors. We found: (1) RF-CM by itself does not induce colony formation. (2) In the presence of erythropoietin, RF-CM enhances the growth of BFU-E. (3) Recombinant IL 4 also enhances BFU-E formation, but in our assays IL 4 induced fewer colonies than RF-CM and the colonies were smaller. (4) Because neither IL 4 nor RF-CM, by themselves, can stimulate colony formation, we compared the effect of RF-CM on assays that are known to show other IL 4 functions. RF-CM did not induce proliferation of PHA induced blast T cells, a known property of IL 4.(ABSTRACT TRUNCATED AT 250 WORDS)

L27 ANSWER 37 OF 40 MEDLINE
ACCESSION NUMBER: 85266058 MEDLINE
DOCUMENT NUMBER: 85266058 PubMed ID: 3894792
TITLE: Glomerular cell culture.
AUTHOR: Striker G E; Striker L J
SOURCE: LABORATORY INVESTIGATION, (1985 Aug) 53 (2) 122-31.
Ref: 92
Journal code: 0376617. ISSN: 0023-6837.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198509
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850916

AB Glomerular cell culture has now become a widely used research technique. At the present time procedures are available to obtain **isolated** glomeruli from nearly all species. The **isolation** of individual cells has proven problematical. This is due to the lack of defined markers. Thus, it is not yet possible to determine the presence and relative degree of contamination by other glomerular or even nonglomerular, cell types. The importance of dealing with individual cell types, or defined mixtures, is exemplified by the variable results obtained in the assessment of prostaglandin synthesis within and between species. Several important bits of information have, nevertheless, evolved from glomerular cell culture experiments. The sites of synthesis of basement membrane components, as well as their composition, have been determined. Confirmation of the existence of a **bone marrow**-derived mesangial cell population and some of their properties has been obtained. The response of mesangial cells to, as well as their **production** of, various mediators has been shown. Finally, clear evidence for interspecies differences and similarities has been documented. Areas of controversy remain, including whether contractile mesangial cells are phagocytic, the presence of C3b receptors on epithelial cells, the amounts and types of certain extracellular matrix products synthesized by the various cell types, and the best methods for separation and culture of the individual glomerular cell types. There remain many fruitful areas for research. Fundamental questions such as the appropriate basal medium and supplements, the type of **substrate**, and the means to separate the individual cell types remain as unanswered or partially answered questions. When **isolated** cells are reliably obtained, the study of biosynthetic products in the resting and stimulated states must be again addressed. At that point, the effect of various and deliberate combinations of the glomerular cell types on the biosynthetic or proliferative responses will require further studies. For instance, although contractility mediated by receptors for angiotensin II has been assumed to be a specific property of mesangial cells, recent work shows that epithelial cells also respond to angiotensin II. In addition, the handling of immune complexes by various cells needs to be further investigated (43). Similarly, the pharmacologic response of the diverse populations of glomerular cells represents another area of study that has just begun. Finally, these data will provide the backdrop on which the analysis of various induced and genetic diseases can be performed.(ABSTRACT TRUNCATED AT 400 WORDS)

L27 ANSWER 38 OF 40 MEDLINE
ACCESSION NUMBER: 85192238 MEDLINE
DOCUMENT NUMBER: 85192238 PubMed ID: 6533755
TITLE: Metabolic aspects of cell cycle regulation in normal and cancer cells.
AUTHOR: Olivotto M; Arcangeli A; Caldini R; Chevanne M; Cipolleschi M G; Dello Sbarba P
SOURCE: TOXICOLOGIC PATHOLOGY, (1984) 12 (4) 369-73.
Journal code: 7905907. ISSN: 0192-6233.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198506
ENTRY DATE: Entered STN: 19900320
Last Updated on STN: 19900320
Entered Medline: 19850620

AB Several studies are reviewed dealing with the mechanisms which regulate the cell cycle progression in normal and cancer cells. Using Yoshida AH 130 ascites tumor cells, it has been found that the G1-S transition of these cells is impaired by specific inhibitors of the electron flow through the respiratory chain (antimycin A), although respiratory ATP can be replaced by glycolytic ATP. The above transition can be also inhibited by the addition of physiologic **substrates**, mainly pyruvate, by a mechanism which appears linked to a modification of the cellular redox state and can be totally reversed by adding adenine to the **culture medium**. Adenine equally **removes** the block **produced** by antimycin A, pointing out a respiration-linked step of purine metabolism restricting the cell recruitment into S. A substantial protection of this step against the inhibitory effects of pyruvate and antimycin A has been obtained by the addition of folate and tetrahydrofolate, suggesting that the respiration-linked limiting step of tumor cell cycling involves folate metabolism and its connection to purine synthesis. The biologic relevance of these findings is stressed by the fact that pyruvate addition also inhibits the proliferation of concanavalin A-stimulated lymphocytes as well as of **bone marrow** hemopoietic cells in the presence of colony-stimulating factors. On the other hand, pyruvate only slightly affects the growth kinetics of malignant lymphoblasts and of Friend erythroleukemia cells either in the absence or in the presence of the differentiation inducer dimethylsulfoxide.

L27 ANSWER 39 OF 40 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 84170455 MEDLINE
DOCUMENT NUMBER: 84170455 PubMed ID: 6324225
TITLE: Characterization of cells **isolated** and cultured from human **bone.**
AUTHOR: Wergedal J E; Baylink D J
CONTRACT NUMBER: AM 31062 (NIADDK)
SOURCE: PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, (1984 May) 176 (1) 60-9.
Journal code: 7505892. ISSN: 0037-9727.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198405
ENTRY DATE: Entered STN: 19900319
Last Updated on STN: 19970203
Entered Medline: 19840507

AB Cells **isolated** from samples of human iliac crest and human femoral heads by collagenase digestion have been successfully cultured in Fitton-Jackson modified BGJb **culture medium** supplemented with penicillin (100 units/ml), streptomycin (100 micrograms/ml), and fetal calf serum (10%).

Although only a low proportion of the cells survived the initial plating (less than 1%), cells established in culture were readily passaged. Examination of cells obtained at intervals during the collagenase digestion showed that the percentage of cells that attached increased with time of digestion. Rapid sample **preparation** of rat **bone** did not substantially increase the number of cells attaching. Thus, it seems unlikely that the low survival was due to loss of viability during sample transportation and **preparation**. Of several media tested BGJb supplemented with 10% fetal calf serum supported the best growth. Population doubling time averaged 104 hr. Cultured human **bone** cells were assayed for alkaline phosphatase activity using the azo dye method with naphthol ASTR phosphate as the **substrate**. A portion of the cells (19%) demonstrated high activity in all cultures examined regardless of the passage number of the culture. Autoradiography of cells exposed to [3H]thymidine showed incorporation of the label into both alkaline phosphate-positive and -negative cells. The stimulation of cell proliferation by **growth factors** was studied by determining the incorporation of [3H]thymidine into DNA. The specific skeletal **growth factor** from human **bone** stimulated cell proliferation several-fold with a half-maximal effect at 5 micrograms/ml. Insulin, epidermal **growth factor**, and a crude **preparation** of somatomedin C also stimulated cell proliferation.

L27 ANSWER 40 OF 40 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 79197516 MEDLINE
DOCUMENT NUMBER: 79197516 PubMed ID: 376921
TITLE: Growth of normal and malignant human mammary epithelial cells in culture.
AUTHOR: Kirkland W L; Yang N S; Jorgensen T; Longley C; Furmanski P
SOURCE: JOURNAL OF THE NATIONAL CANCER INSTITUTE, (1979 Jul) 63 (1) 29-41.
Journal code: 7503089. ISSN: 0027-8874.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197909
ENTRY DATE: Entered STN: 19900315
Last Updated on STN: 19900315
Entered Medline: 19790901

AB Normal and malignant human mammary epithelial cells were placed in culture. Normal cells were recovered from late-lactation milk and breast fluids, and malignant cells were isolated from primary breast tumors by collagenase digestion. The concentration of cells obtained from breast fluid samples was inversely proportional to the volume of fluid secreted. Most of these cells adhered rapidly to the **substrate**, did not replicate, displayed Fc receptor-dependent phagocytic activity, and were thus identified as macrophages. The remaining cells grew out into large islands comprised of one or two distinct morphologic types of mammary epithelial cells. Optimum growth of these cells was obtained in medium buffered to pH 6.8, and the epidermal **growth factor** markedly prolonged the exponential growth phase of the cells. Two morphologically distinct populations of epithelial

cells were also observed in cultures established from individual breast tumors. Growth of the malignant cells was relatively unaffected by the pH of the **culture medium**, and the cells were unresponsive to exogenously added hormones. Overgrowth of malignant epithelial cells in primary cultures by **stromal** fibroblasts was retarded by replacement of standard growth medium with fresh medium containing a serum substitute; growth of the malignant epithelial cells was unaffected. A feeder layer of mitomycin C-treated human fibroblasts increased the plating efficiency of both normal and malignant cells in primary culture and also facilitated passage of these cells to secondary and tertiary cultures.

(FILE 'MEDLINE' ENTERED AT 11:22:02 ON 23 JUN 2003)

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L29         4250 SEA FILE=MEDLINE ABB=ON  PLU=ON  "STROMAL CELLS"/CT
L30          123 SEA FILE=MEDLINE ABB=ON  PLU=ON  L28 AND L29
L31        74305 SEA FILE=MEDLINE ABB=ON  PLU=ON  "CULTURE MEDIA"/CT
L32            5 SEA FILE=MEDLINE ABB=ON  PLU=ON  L30 AND L31


L28        19650 SEA FILE=MEDLINE ABB=ON  PLU=ON  "GROWTH SUBSTANCES"/CT
L31        74305 SEA FILE=MEDLINE ABB=ON  PLU=ON  "CULTURE MEDIA"/CT
L33        43231 SEA FILE=MEDLINE ABB=ON  PLU=ON  "BONE AND BONES"/CT
L34          276 SEA FILE=MEDLINE ABB=ON  PLU=ON  L28 AND L33
L35           14 SEA FILE=MEDLINE ABB=ON  PLU=ON  L34 AND L31


L36          19 L32 OR L35
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L36 ANSWER 1 OF 19 MEDLINE
AN 2002215801 MEDLINE
TI Preparation of neural progenitors from bone marrow and umbilical cord blood.
AU Song Shijie; Sanchez-Ramos J
SO METHODS IN MOLECULAR BIOLOGY, (2002) 198 79-88.
Journal code: 9214969. ISSN: 1064-3745.

L36 ANSWER 2 OF 19 MEDLINE
AN 2001075194 MEDLINE
TI [Contribution of in vitro models (cell cultures and growth factors)].
Apport des modeles in vitro (cultures cellulaires et facteurs de croissance).
AU Heymann D
SO REVUE DE CHIRURGIE ORTHOPEDIQUE ET REPARATRICE DE L APPAREIL MOTEUR, (2000 Sep) 86 Suppl 1 152-3.
Journal code: 1272427. ISSN: 0035-1040.

L36 ANSWER 3 OF 19 MEDLINE
AN 1998455294 MEDLINE
TI Androgen responsiveness of stromal cells of the human prostate: regulation of cell proliferation and keratinocyte growth factor by androgen.
AU Planz B; Wang Q; Kirley S D; Lin C W; McDougal W S
SO JOURNAL OF UROLOGY, (1998 Nov) 160 (5) 1850-5.

Journal code: 0376374. ISSN: 0022-5347.

AB PURPOSE: Growth and development of the prostate are androgen dependent and mainly influenced by stromal-epithelial interaction. It is believed that indirect androgenic activation of paracrine factors like keratinocyte growth factor (KGF) in the prostatic stroma influences the growth of epithelial cells. In this study we investigated the role androgen plays in stromal cell growth and stimulation of KGF in the human prostate. MATERIALS AND METHODS: Stromal cells were derived from explant primary culture of human normal or benign prostatic tissue. The effect of different dihydrotestosterone (DHT) concentrations on cell proliferation was measured using 3[H]thymidine incorporation assay. The effect of DHT on levels of KGF protein was determined by Western blotting. The effect of DHT on levels of KGF gene expression was measured by various cycles of polymerase-chain-reaction (PCR) and multiplex PCR. RESULTS: Characterization of stromal cells showed epithelial cells less than 9.5% in all passages. DHT stimulated human prostate stromal cells in a dose dependent fashion over a concentration range of 0.001-10 nM. Immunocytochemical evaluation of KGF after DHT exposure showed a higher staining intensity. Relative quantitation of Western blotting showed a 1.93-fold increase in KGF protein in the androgen treated stromal cells. At 1 nM DHT conventional and multiplex PCR revealed a significant stimulation of the KGF mRNA expression. CONCLUSIONS: These data show for the first time that androgen stimulates cell proliferation as well as KGF protein and gene expression in human prostate stromal cells. This supports the hypothesis that androgen-induced stromal-derived KGF stimulates prostate epithelial cell growth.

L36 ANSWER 4 OF 19 MEDLINE

AN 1998006451 MEDLINE

TI Modulation of commitment, proliferation, and differentiation of chondrogenic cells in defined culture medium.

AU Quarto R; Campanile G; Cancedda R; Dozin B

SO ENDOCRINOLOGY, (1997 Nov) 138 (11) 4966-76.
Journal code: 0375040. ISSN: 0013-7227.

AB The factors regulating the growth and development of mesenchymal precursor cells toward chondrogenesis are not well identified. We have developed a defined serum-free culture system that allows the proliferation of chick embryo chondrogenic cells and their maturation toward hypertrophic chondrocytes. Proliferation is obtained in adhesion in medium supplemented with insulin (Ins), Dexamethasone (Dex), and either basic fibroblast growth factor (FGF-2), platelet-derived growth factor bb, epithelial growth factor, or GH; the highest mitogenic response is induced by FGF-2 in synergy with Ins. Ins can be substituted by Ins-like growth factor I. When these cells are transferred into suspension culture in Ins/Dex and T3 without growth factor supplement, they undergo the complete chondrogenic development characterized by type X collagen synthesis and cellular hypertrophy. During differentiation, Ins cannot be substituted by Ins-like growth factor I. Chondrogenesis is also evidenced by the formation of hypertrophic cartilage when the medium is supplemented with ascorbic acid. If T3 is introduced in the proliferation phase, the cells fail to differentiate to hypertrophy in suspension unless bone morphogenetic protein-2 is added. Assays of ectopic tissue formation in nude mice, with cells implanted sc after adsorption on collagen sponge or porous hydroxyapatite ceramics, indicate that cells grown in Ins/FGF-2

reform mainly cartilage in vivo, whereas expansion in Ins/T3/Dex/FGF-2 leads to the formation of cartilage, bone, and adipose tissue.

L36 ANSWER 5 OF 19 MEDLINE
AN 97464476 MEDLINE
TI The role of soluble growth factors in inducing transient growth and clonal extinction of stroma cell dependent erythroblastic leukemia cells.
AU Itoh K; Friel J; Laker C; Zeller W; Just U; Bittner S; Nibbs R J; Harrison P R; Nishikawa S I; Mori K J; Ostertag W
SO LEUKEMIA, (1997 Oct) 11 (10) 1753-61.
Journal code: 8704895. ISSN: 0887-6924.
AB A coculture system of a murine erythroblastic leukemia cell line (ELM-D) with its supportive stromal cell line (MS-5) was established. Long-term growth of ELM-D cells is strictly stroma cell dependent. Interaction between stem cell factor (SCF) and its receptor, c-kit, was demonstrated to be important for stroma cell-dependent growth by anti c-kit neutralizing monoclonal antibody (mAb) inhibition experiments. Significantly, soluble growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3) or SCF of MS-5 stromal cells (MS-5 CM) could replace the requirement of stroma cells for a considerable period. However, ELM-D cells maintained in these growth factors underwent clonal extinction after 3-6 weeks unless contact with stroma was re-established. Furthermore, IL-3 or GM-CSF acted in a dominant manner in inducing cell death in the presence of stroma cells. Cells showing clonal extinction undergo programmed cell death and do not differentiate. These altered growth properties of ELM-D cells exposed to soluble growth factors or to stroma cells appear to be analogous to those described for T or B cells primed by antigen presenting cells and then grown in growth factors.

L36 ANSWER 6 OF 19 MEDLINE
AN 97141691 MEDLINE
TI Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in the human fallopian tube.
AU Keltz M D; Attar E; Buradagunta S; Olive D L; Kliman H J; Arici A
SO AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, (1996 Dec) 175 (6) 1611-9.
Journal code: 0370476. ISSN: 0002-9378.
AB OBJECTIVE: The fallopian tube is the site of fertilization and early embryonic growth and a common site of ectopic implantation. Although the factors responsible for early embryogenesis and implantation are incompletely understood, leukemia inhibitory factor may have an important role in early embryonic development and implantation. We set out to evaluate the production and modulation of leukemia inhibitory factor in the fallopian tube. STUDY DESIGN: We first investigated leukemia inhibitory factor messenger ribonucleic acid levels in fallopian tubes. We then investigated leukemia inhibitory factor messenger ribonucleic acid and protein production in tubal epithelial and stromal cell cultures. RESULTS: Leukemia inhibitory factor messenger ribonucleic acid is expressed in the fallopian tube with only slight variation during the menstrual cycle; however, it is markedly elevated in association with ectopic pregnancy. The level is higher in the tubal mucosa than in the remaining layers and is higher in the more distal segments of the fallopian tube. Estradiol and progesterone did not

modulate leukemia inhibitory factor expression in epithelial or stromal cell cultures. Interleukin-1 alpha, tumor necrosis factor-alpha, and transforming growth factor-beta enhanced leukemia inhibitory factor expression in epithelial and stromal cells, with transforming growth factor-beta 1 enhancing expression by fourfold in stromal cells. Epithelial cells secreted high levels of leukemia inhibitory factor compared with stromal cells (332 +/- 89 vs 25 +/- 42 pg/mg total protein). Yet stromal cells treated with transforming growth factor-beta alone or in combination with epidermal growth factor and platelet-derived growth factor, as well as TNF-alpha alone or in combination with interleukin-1 alpha enhanced secretion of leukemia inhibitory factor at or above the levels found with epithelial cells. CONCLUSIONS: We speculate that the high constitutive levels of leukemia inhibitory factor expressed in the ampullary portion of the fallopian tube may play a role in early embryonic development. Additionally, elevated expression with ectopic implantation and the marked induction of secretion in the tubal stroma by growth factors and cytokines suggest a link between inflammation, leukemia inhibitory factor, and tubal ectopic pregnancies.

L36 ANSWER 7 OF 19 MEDLINE
AN 97107143 MEDLINE
TI Growth factor control of cultured rat uterine stromal cell proliferation is progesterone dependent.
AU Piva M; Flieger O; Rider V
SO BIOLOGY OF REPRODUCTION, (1996 Dec) 55 (6) 1333-42.
Journal code: 0207224. ISSN: 0006-3363.
AB Uterine stromal cells undergo mitosis and differentiate into the decidua just prior to the expected time of implantation in humans and rodents. We have utilized a culture system that will be suitable for study of the molecular mechanisms regulating stromal cell proliferation. Stromal cells were isolated from the uteri of ovariectomized rats and were cultured in chemically defined medium. Cultured cells express the mesenchymal markers vimentin and desmin. They do not express the epithelial marker cytokeratin. Serum-starved stromal cells were stimulated to proliferate in a time frame consistent with the cell cycle through addition of a panel of growth factors (basic fibroblast growth factor [bFGF], epidermal growth factor, platelet-derived growth factor, transforming growth factor alpha, insulin-like growth factor I) and hormones to the culture medium. None of the growth factors tested significantly stimulated proliferation in the absence of progesterone. Furthermore, progesterone was the only steroid of those tested that stimulated mitosis in the presence of growth factors. Stromal cell proliferation in response to progesterone and bFGF was dose dependent and saturable. Addition of the progesterone receptor antagonist mifepristone (RU486) and an inhibitor of tyrosine kinase receptor activation (suramin) abolished stromal cell mitosis. Progesterone receptors and fibroblast growth factor receptor 1 (FGFR1) were identified by immunoblot analysis in proliferating stromal cells. Taken together, these results show that cultured stromal cells maintain progesterone-dependent cell cycle control that is mediated via progesterone receptors. Moreover, the data indicate that bFGF control of stromal cell proliferation is modulated via a specific isoform of FGFR1 containing the three-loop immunoglobulin-like domain.

L36 ANSWER 8 OF 19 MEDLINE
AN 92351810 MEDLINE
TI Factors influencing synthesis and mineralization of bone matrix from fetal bovine bone cells grown in vitro.
AU Whitson S W; Whitson M A; Bowers D E Jr; Falk M C
SO JOURNAL OF BONE AND MINERAL RESEARCH, (1992 Jul) 7 (7) 727-41.
Journal code: 8610640. ISSN: 0884-0431.
AB This study of the in vitro synthesis and mineralization of bovine bone demonstrates that sheets of mineralized matrix can be produced consistently within 18-24 days of cell isolation. Mineralization surpasses that achieved by other systems with other species: The deposition of mineral extends beyond nodules to form branching trabeculae and then solid wafers of bone. Comparison of the fetal age of the bone source, enzyme digestion methods, seeding density, culture surface, nutritive media, and concentration of fetal calf serum and other additives, including insulin and ascorbic acid, has yielded a set of optimal culture conditions. In the presence of ascorbic acid and beta-glycerol phosphate, insulin has a dose-dependent effect on the morphology of the mineralized bone matrix produced. Quantitative analysis shows that in these cultures calcium accumulates most rapidly between days 6 and 10 after the introduction of mineralization medium but that mineral accretion continues throughout 14-16 days of culture. Alkaline phosphatase levels rise up to 200-fold, concomitant with a rapid increase in the number of cells per culture during the early mineralization phases; both fall as mineralization proceeds. This system has been used to study the induction of mRNA of type I collagen, alkaline phosphatase, and several noncollagenous bone proteins during the course of mineralization. Because of the degree of mineralization achieved with this system, it has many potential applications.

L36 ANSWER 9 OF 19 MEDLINE
AN 92244630 MEDLINE
TI Mitogenic and dentin-inductive effects of crude bone morphogenetic protein from bone and dentin in primary adult pulp cell culture.
AU Nakashima M
SO ORAL SURGERY, ORAL MEDICINE, AND ORAL PATHOLOGY, (1992 Apr) 73 (4) 484-9.
Journal code: 0376406. ISSN: 0030-4220.
AB The effects of crude bone morphogenetic protein (BMP) derived from bone and dentin matrix on proliferation, production of extracellular matrix, and biologic function of the pulp cell were examined in the primary cell culture from permanent dental pulp. BMP from bone and from dentin matrix stimulated iodine 125-deoxyuridine incorporation in the absence of 10% calf serum. They increased sulfur 35-sulfate incorporation in proliferating stage and had no effects in stationary stage of culture. Alkaline phosphatase activities were inhibited in proliferating, stationary, and multilayered stages of culture. Osteocalcin synthesis was increased in culture treated with BMP from day 2 to day 10. These findings suggest that crude BMP might have mitogenic activity and some role in regulation of differentiation of pulp cells into odontoblasts.

L36 ANSWER 10 OF 19 MEDLINE
AN 92203416 MEDLINE
TI Prostate and bone fibroblasts induce human prostate cancer growth in vivo: implications for bidirectional tumor-stromal cell interaction in prostate carcinoma growth and metastasis.

AU Gleave M E; Hsieh J T; von Eschenbach A C; Chung L W
SO JOURNAL OF UROLOGY, (1992 Apr) 147 (4) 1151-9.
Journal code: 0376374. ISSN: 0022-5347.
AB Prostate cancer selectively metastasizes to the axial skeleton to produce osteoblastic lesions, which suggests that bidirectional paracrine interactions exist between prostate cancer and bone cells. To evaluate the role of tumor-stromal cell interaction and stromal-specific growth factors in prostate cancer growth and dissemination, we coinoculated nontumorigenic human prostate cancer cells (LNCaP) and various tissue-specific fibroblasts subcutaneously in athymic mice. LNCaP tumors were induced most consistently by human bone fibroblasts (62%), followed by two prostate fibroblast cell lines (31% and 17%), but not by lung, kidney, or embryonic 3T3 fibroblasts. Carcinomas formed preferentially in male hosts, demonstrating in vivo androgen sensitivity. Immunohistochemical and biochemical techniques confirmed the human prostate component of these tumors and were paralleled by elevations in serum prostate specific antigen. In vitro mitogenic assays revealed a two-to three-fold bidirectional stimulation between LNCaP and bone or prostate fibroblast conditioned media, but not lung, kidney, or 3T3 fibroblast conditioned media. A novel method developed to deliver concentrated bone or prostate fibroblast conditioned media in vivo using a slowly absorbed matrix (gelfoam) also induced tumor formation, emphasizing the importance of fibroblast growth factors in LNCaP tumor formation. Northern analysis identified the stromal compartment as the primary source of extracellular matrix (collagen, fibronectin), while only LNCaP cells expressed transforming growth factor alpha. Although LNCaP and stromal cells express basic fibroblast growth factor (bFGF), the bidirectional paracrine-mediated mitogenic activity between these cells is not inhibited by anti-bFGF antibodies, suggesting that other undefined growth factors may be involved in stimulating LNCaP growth. These observations illustrate the importance of stromal-epithelial interaction in prostate tumor growth and suggest that extracellular matrix and paracrine-mediated growth factors play a role in prostate cancer growth and metastasis.

L36 ANSWER 11 OF 19 MEDLINE
AN 92033315 MEDLINE
TI Osteoclast growth factor activity in medium conditioned by fetal rat bones.
AU Scheven B A; Hamilton N J; Duncan A; Robins S P
SO BONE AND MINERAL, (1991 Sep) 14 (3) 221-35.
Journal code: 8610542. ISSN: 0169-6009.
AB The presence and biological activity of an Osteoclast Growth Factor (OGF) was investigated in serum-free medium conditioned by periostless fetal rat calvaria in culture. OGF activity was assessed using in vitro systems of fetal rat long bones and adult rat bone marrow cells. Rat calvaria conditioned medium (RCCM) increased the number of osteoclasts in the long bone cultures, partly due to stimulation of progenitor proliferation. RCCM did not exert a direct bone-resorbing activity (45Calcium release assay) on the pre-existing osteoclasts residing in the long bones, but stimulated bone resorption in long term cultures, apparently in an indirect manner by enhancing the number of osteoclasts. In cultures of bone marrow cells isolated from adult rats, RCCM markedly stimulated the formation of mononuclear cells which were positively stained for tartrate-resistant acid phosphatase (TRAP). The

osteoclastic nature of the cells was confirmed by specific labeling with 125I-calcitonin. Formation of the TRAP-positive cells was significantly inhibited by salmon calcitonin. CM from fetal rat skin cultures did not display a significant OGF activity. Furthermore, unlike the bone marrow cells, peritoneal macrophages did not respond to RCCM and remained devoid of TRAP activity. Neutralization experiments with a specific antibody to GM-CSF indicated that OGF activity in the RCCM could not be ascribed to this hemopoietic growth factor. Secretion of OGF activity was mainly dependent on protein synthesis as addition of cycloheximide to the calvaria cultures significantly inhibited the secretion of OGF into the medium. G3000 HPLC fractionation of RCCM revealed two major OGF peaks with Mr 14,000 and 70,000. Two subsequent reverse-phase HPLC steps using the lower Mr OGF fraction led to a highly purified OGF fraction. The results of this study further provide evidence that bone tissue produces factor(s) which specifically govern the process of osteoclast development, thus providing information about one of the mechanisms controlling bone resorption.

L36 ANSWER 12 OF 19 MEDLINE
AN 91228703 MEDLINE
TI Longitudinal bone growth in vitro: effects of insulin-like growth factor I and growth hormone.
AU Scheven B A; Hamilton N J
SO ACTA ENDOCRINOLOGICA, (1991 May) 124 (5) 602-7.
Journal code: 0370312. ISSN: 0001-5598.
AB Longitudinal growth was studied using an in vitro model system of intact rat long bones. Metatarsal bones from 18- and 19-day-old rat fetuses, entirely (18 days) or mainly (19 days) composed of chondrocytes, showed a steady rate of growth and radiolabelled thymidine incorporation for at least 7 days in serum-free media. Addition of recombinant human insulin-like growth factor-I to the culture media resulted in a direct stimulation of the longitudinal growth. Recombinant human growth hormone was also able to stimulate bone growth, although this was generally accomplished after a time lag of more than 2 days. A monoclonal antibody to IGF-I abolished both the IGF-I and GH-stimulated growth. However, the antibody had no effect on the growth of the bone explants in control, serum-free medium. Unlike the fetal long bones, bones from 2-day-old neonatal rats were arrested in their growth after 1-2 days in vitro. The neonatal bones responded to IGF-I and GH in a similar fashion as the fetal bones. Thus in this study in vitro evidence of a direct effect of GH on long bone growth via stimulating local production of IGF by the growth plate chondrocytes is presented. Furthermore, endogenous growth factors, others than IGFs, appear to play a crucial role in the regulation of fetal long bone growth.

L36 ANSWER 13 OF 19 MEDLINE
AN 91004059 MEDLINE
TI Human prostatic cancer cells, PC3, elaborate mitogenic activity which selectively stimulates human bone cells.
AU Perkel V S; Mohan S; Herring S J; Baylink D J; Linkhart T A
SO CANCER RESEARCH, (1990 Nov 1) 50 (21) 6902-7.
Journal code: 2984705R. ISSN: 0008-5472.
AB Prostatic cancer typically produces osteoblastic metastases which are not attended by marrow fibrosis (i.e., osteoblast but not stromal fibroblast proliferation). In the present study we sought

to test the hypothesis that prostatic cancer cells produce factor(s) which act selectively on human osteoblasts. Such a paracrine mechanism would explain the observed increase in osteoblasts, unaccompanied by an increase in marrow fibroblasts. To test this hypothesis we investigated the mitogenic activity released by the human prostatic tumor cell line, PC3. PC3 cells have been reported previously to produce mitogenic activity for cells that was relatively specific for rat osteoblasts compared to rat fibroblasts. However, the effects of this activity on human cells has not been examined previously. PC3-conditioned medium (CM) (5-50 micrograms CM protein/ml) stimulated human osteoblast proliferation by 200-950% yet did not stimulate human fibroblast proliferation [(3H]thymidine incorporation). PC3 CM also increased cell numbers in human osteoblast but not fibroblast cell cultures. To determine whether the osteoblast-specific mitogenic activity could be attributed to known bone growth factors, specific assays for these growth factors were performed. PC3 CM contained 10 pg insulin-like growth factor (IGF) I, less than 2 pg IGF II, 54 pg basic fibroblast growth factor, and 16 pg transforming growth factor beta/microgram CM protein. None of these growth factors alone or in combination could account for the observed osteoblast-specific PC3 cell-derived mitogenic activity. Furthermore, when 5 micrograms/ml PC3 CM was tested in combination with maximally effective concentrations of either basic fibroblast growth factor, IGF I, IGF II, or transforming growth factor beta, it produced an additive effect suggesting that PC3 CM stimulates osteoblast proliferation by a mechanism independent of these bone mitogens. Biochemical characterization supported the hypothesis that the PC3 cell growth factor was unique from other growth factors. The PC3 growth factor did not bind to heparin and was resistant to acid as well as the reducing agent, dithiothreitol. Sephadex G-75 and fast protein liquid chromatography Mono S cation-exchange chromatography revealed the PC3-derived mitogen to be an Mr 26,000-30,000 basic protein. Therefore, we conclude that PC3 cells release a mitogen which exhibits higher specificity for human osteoblasts than human fibroblasts and is unique from other growth factors tested. Production of this mitogen by human prostatic carcinoma cells could play an etiological role in the intense osteoblast-specific stimulation that occurs at sites of bone metastases.

L36 ANSWER 14 OF 19 MEDLINE
AN 90200401 MEDLINE
TI Further characterization of osteogenic-cell growth promoting activity derived from healing bone marrow.
AU Gazit D; Shteyer A; Bab I
SO CONNECTIVE TISSUE RESEARCH, (1989) 23 (2-3) 153-61.
Journal code: 0365263. ISSN: 0300-8207.
AB During its osteogenic phase, post-ablation regenerating bone marrow produces bone promoting activity to osteogenic cells. In the experiments reported, activity derived from (rat) healing bone marrow conditioned medium (HBMCM) after boiling was analyzed using chromatography on heparin-Sepharose. The activity in HBMCM was shown to be divided among at least six independent activities that stimulated DNA synthesis rates is osteogenic rat osteosarcoma (ROS) cells. Three activities resolved when heparin-Sepharose was washed isocratically with phosphate buffered saline. Two of these were resistant to reduction and acidification and their effect was considerably more potent in osteogenic than non-osteogenic ROS

cells. Three additional activity peaks recovered when the heparin-Sepharose column was pumped with an NaCl gradient. Two of them eluted at 0.3 and 0.65 M NaCl, affected osteogenic and non-osteogenic ROS cells to a similar extent and may be attributed to platelet-derived growth factor. A third peak, resolved at 1.2 M NaCl, implies the residual activity of acidic fibroblast growth factor that persisted after boiling of the conditioned medium. It is concluded that the activity profile of HBMCM reflects the in vivo situation where the osteogenic phase of marrow regeneration is probably regulated by multiple growth factor species.

L36 ANSWER 15 OF 19 MEDLINE
AN 89102705 MEDLINE
TI Effects of transforming growth factor type beta upon bone cell populations grown either in monolayer or semisolid medium.
AU Guenther H L; Cecchini M G; Elford P R; Fleisch H
SO JOURNAL OF BONE AND MINERAL RESEARCH, (1988 Jun) 3 (3) 269-78. Journal code: 8610640. ISSN: 0884-0431.
AB Bone has been shown to store large amounts of transforming growth factor type beta (TGF beta) and this has recently been found to be synthesized by bone-forming cells. We report on studies undertaken to examine the effects of platelet-derived TGF beta on different bone cell populations, isolated from 1-day postnatal rat calvaria by sequential enzymatic digestion. In addition, we tried to determine which of these cell populations synthesize TGF beta. In this regard, evidence was collected to indicate that cell populations which were shown to be enriched with osteoblast-like cells synthesize TGF beta. Although the production of the factor appeared to be limited to a particular cell type, its action was found to be of a more general character, as all cell populations were found to respond to TGF beta. Contrary to earlier reports, TGF beta was shown to be inhibitory upon cell proliferation. In this context, growth of cells released during early digestions was reduced considerably more than growth of those released during late digestions. Studies on the effect upon protein synthesis revealed that TGF beta specifically inhibited collagen but not the synthesis of noncollagenous proteins. The synthesis of collagen was altered to a greater extent in cells isolated during late digestions than in cells of the early populations. Further information on the TGF beta-mediated effects on bone cell biology was provided by data showing that both alkaline phosphatase and cAMP production in response to PTH was greatly reduced by TGF beta. Finally, experiments performed to determine whether TGF beta induces any of the bone cell populations to acquire the transformed phenotype revealed that only populations previously shown to be enriched with osteoblast-like cells formed colonies in soft agarose.(ABSTRACT TRUNCATED AT 400 WORDS)

L36 ANSWER 16 OF 19 MEDLINE
AN 86192132 MEDLINE
TI Isolation of a nontransforming bone-derived growth factor from medium conditioned by fetal rat calvariae.
AU Canalis E; Centrella M
SO ENDOCRINOLOGY, (1986 May) 118 (5) 2002-8. Journal code: 0375040. ISSN: 0013-7227.
AB Previous studies have indicated that medium conditioned by 21-day-old fetal rat calvariae contains bioactive proteins termed bone-derived growth factors (BDGF) I and II. In the present studies

we have purified the nontransforming BDGF II by dialysis, molecular sieving, three reverse phase HPLC steps, and preparative polyacrylamide gel electrophoresis. The second HPLC step (HPLC-2) yielded a recovery of 22% of the biological activity and achieved a 1500-fold purification, resulting in 20 micrograms protein/liter calvarial conditioned medium; the third HPLC step was of limited value in the purification of BDGF. Analytical PAGE revealed that the majority of the protein in HPLC-2-purified BDGF migrated with a relative molecular mass (Mr) of 11,000 and two additional proteins were seen at a Mr of 22,000-23,000. On preparative PAGE, the material migrating with a Mr of 11,000 stimulated parameters of bone and fibroblast growth in vitro, whereas the material with a Mr of 22,000-23,000 had less biological activity. Isoelectric focusing revealed that BDGF had an isoelectric point (pI) of 5. BDGF enhanced the incorporation of [3H]thymidine into DNA in fibroblast and calvarial cultures and of [3H]proline into collagen and noncollagen protein in calvariae. In conclusion, fetal rat calvariae secrete a BDGF with an estimated Mr of 11,000 and a pI of 5; this material stimulates bone and fibroblast growth in vitro.

L36 ANSWER 17 OF 19 MEDLINE
AN 85200024 MEDLINE
TI A mouse tumor-derived osteolytic factor stimulates bone resorption by a mechanism involving local prostaglandins production in bone.
AU Lau K H; Lee M Y; Linkhart T A; Mohan S; Vermeiden J; Liu C C; Baylink D J
SO BIOCHIMICA ET BIOPHYSICA ACTA, (1985 May 29) 840 (1) 56-68. Journal code: 0217513. ISSN: 0006-3002.
AB Culture medium which was conditioned by tissue of a CE mouse breast tumor in vitro contained dose-dependent osteolytic activity. The osteolytic activity was not soluble in dichloromethane and ethylacetate, indicating that it was not attributable to vitamin D metabolites or prostaglandins. However, breast tumor-conditioned medium stimulated production and release of prostaglandin E2 from mouse calvaria in vitro, and the stimulation of bone resorption in vitro by breast tumor-conditioned medium was blocked by a dose of indomethacin that prevented stimulation of mouse calvarial prostaglandin E2 production and release. The resorptive activity of parathyroid hormone (PTH) was not affected by the same dose of indomethacin, suggesting that the osteolytic factor was not PTH. This was further supported by observation that mouse kidney cell cAMP production was stimulated by PTH, but not by the aqueous phase of ethylacetate-extracted breast tumor-conditioned medium. In addition to osteolytic activity, breast tumor-conditioned medium contained a dose-dependent bone cell mitogenic activity, demonstrated by the stimulation of [3H]thymidine incorporation into trichloroacetic acid-insoluble macromolecules and a corresponding increase in bone cell number in monolayer cultures of bone cells. Breast tumor-conditioned medium also contained a dose-dependent transforming growth factor-(TGF-) like activity as defined by its ability to transform anchorage-dependent growth of nontransformed cells to anchorage-independent growth. The TGF in breast tumor-conditioned medium did not compete with epidermal growth factor (EGF) for EGF receptor binding, but its transforming activity was greatly enhanced by EGF, indicating that it was a beta-type TGF. Both the osteolytic and mitogenic activities were nondialyzable, sensitive to reducing agent, and not removable by dichloromethane and ethylacetate extractions. Furthermore, the TGF activity was not

removed by ethylacetate extraction. Thus, the possibility that these activities in breast tumor-conditioned medium might be mediated by the same molecule must be considered. In summary, our data suggest that the CE mouse mammary carcinoma cells produce and secrete into the culture medium an osteolytic factor which is neither PTH nor prostaglandin and which stimulates local synthesis in bone of prostaglandin E2 which in turn increases bone resorption in vitro.

L36 ANSWER 18 OF 19 MEDLINE
AN 84170455 MEDLINE
TI Characterization of cells isolated and cultured from human bone.
AU Wergedal J E; Baylink D J
SO PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, (1984 May) 176 (1) 60-9.
Journal code: 7505892. ISSN: 0037-9727.
AB Cells isolated from samples of human iliac crest and human femoral heads by collagenase digestion have been successfully cultured in Fitton-Jackson modified BGJb culture medium supplemented with penicillin (100 units/ml), streptomycin (100 micrograms/ml), and fetal calf serum (10%). Although only a low proportion of the cells survived the initial plating (less than 1%), cells established in culture were readily passaged. Examination of cells obtained at intervals during the collagenase digestion showed that the percentage of cells that attached increased with time of digestion. Rapid sample preparation of rat bone did not substantially increase the number of cells attaching. Thus, it seems unlikely that the low survival was due to loss of viability during sample transportation and preparation. Of several media tested BGJb supplemented with 10% fetal calf serum supported the best growth. Population doubling time averaged 104 hr. Cultured human bone cells were assayed for alkaline phosphatase activity using the azo dye method with naphthol ASTR phosphate as the substrate. A portion of the cells (19%) demonstrated high activity in all cultures examined regardless of the passage number of the culture. Autoradiography of cells exposed to [3H]thymidine showed incorporation of the label into both alkaline phosphate-positive and -negative cells. The stimulation of cell proliferation by growth factors was studied by determining the incorporation of [3H]thymidine into DNA. The specific skeletal growth factor from human bone stimulated cell proliferation several-fold with a half-maximal effect at 5 micrograms/ml. Insulin, epidermal growth factor, and a crude preparation of somatomedin C also stimulated cell proliferation.

L36 ANSWER 19 OF 19 MEDLINE
AN 83117875 MEDLINE
TI Experimental dissection of avian and murine tissue interactions using organ culture in a serumless medium free from exogenous (nondefined) factors.
AU Slavkin H C; Honig L S; Bringas P Jr
SO PROGRESS IN CLINICAL AND BIOLOGICAL RESEARCH, (1982) 101 217-28.
Journal code: 7605701. ISSN: 0361-7742.

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L37	8091	SEA FILE=MEDLINE ABB=ON	PLU=ON	BONE DEVELOPMENT/CT
L38	26	SEA FILE=MEDLINE ABB=ON	PLU=ON	L37 AND L31

L39 1 SEA FILE=MEDLINE ABB=ON PLU=ON L38 AND L28

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